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## EVALUATION OF THE OPTIMAL TRUE-ILEAL-DIGESTIBLE LYSINE AND THREONINE REQUIREMENT FOR NURSERY PIGS

*N. A. Lenehan, M. D. Tokach, S. S. Dritz<sup>1</sup>, J. L. Usry<sup>2</sup>, R. D. Goodband  
J. M. DeRouchey, J. L. Nelssen, and C. W. Hastad*

### Summary

A total of 1800 pigs (Exp 1, 360; Exp. 2, 1440) were used in two experiments to evaluate the true ileal digestible (TID) lysine and threonine requirement for 24- to 44-lb pigs. In Exp. 1, there were eight pens per treatment, with five pigs (Genetiporc, initially 23.6 lb and 34 d of age) per pen. Experiment 1 was conducted as a combination of two separate trials to simultaneously examine both the TID lysine and threonine requirement, and hence, determine the appropriate threonine-to-lysine ratio. The first part of the trial consisted of five treatments formulated to contain 0.9, 1.0, 1.1, 1.2, or 1.3% TID lysine, with TID threonine at 66% of lysine. The second part consisted of five treatments formulated to 1.3% TID lysine with increasing TID threonine (0.60, 0.66, 0.73, 0.79, or 0.85%). Other amino acids were formulated to either meet or exceed requirement estimates, thereby ensuring lysine and threonine were first limiting. The highest concentrations of both lysine and threonine (1.3% and 0.85%, respectively) were combined in a single diet, which was used in both trials, to give a total of 10 treatments. From d 0 to 17, ADG and feed efficiency (F/G) improved as TID lysine (quadratic,  $P<0.02$ ) and threonine (ADG, linear,  $P<0.03$ ; F/G, quadratic,  $P<0.04$ ) increased. Regression analysis showed that 95% or more of the maximum response was obtained at a

TID threonine-to-lysine ratio of approximately 64% for ADG and 66% for F/G. In Exp. 2, there were 48 pigs per experimental unit (2 pens sharing a fenceline feeder) and six replications per treatment. Pigs (PIC, 24 lb and 39 d of age) were fed experimental diets containing 1.1% TID lysine (calculated to be less than their requirement estimate), with added L-threonine to give TID threonine concentrations of 0.55, 0.60, 0.66, 0.72, or 0.77% and TID threonine-to-lysine ratios of 50, 55, 60, 65, and 70%. For the 21-d trial, ADG (quadratic,  $P<0.07$ ) and F/G (quadratic,  $P<0.01$ ) improved with increasing TID threonine. The best ADG and F/G were observed at 0.72% TID threonine. Hence, it seems that pigs weighing between 22 and 44 lb require approximately 0.72% TID threonine (0.81% total threonine) when fed 1.1% TID lysine, which corresponds to a TID threonine-to-lysine ratio of 65%, similar to results in Exp. 1. Data from these two studies indicate an optimal TID threonine-to-lysine ratio of approximately 64 to 66% for 24- to 44-lb pigs.

(Key Words: Lysine, Nursery Pigs, Pigs, Threonine.)

### Introduction

There is an increased interest in synthetic threonine supplementation in swine diets because it has become more commercially

<sup>1</sup>Food Animal Health and Management Center.

<sup>2</sup>Anjinomoto-Heartland Lysine, Chicago, IL.

available and economically viable. The current National Research Council (NRC) requirement estimate for true-ileal-digestible lysine and threonine for a 24- to 44-lb pig are 1.01 and 0.63% of the diet, respectively, suggesting a TID threonine-to-lysine ratio of 62%. But lysine and threonine requirement estimates from the NRC are less than those currently being used in commercial production. The objective of these experiments was to determine the optimal ratio of threonine to lysine, and to characterize the optimal use of synthetic threonine in diets to maximize growth performance of the nursery pig.

### Procedures

Pigs (Exp. 1, Genetiporc, Saint Bernard, Quebec, Canada; Exp. 2, PIC, Franklin, KY) were housed in environmentally controlled nurseries. In Exp. 1, each pen had slatted metal flooring and contained a stainless-steel self-feeder and one nipple waterer to allow ad libitum consumption of feed and water. In Exp. 2, pens had slatted plastic flooring and contained a stainless-steel self-feeder and one cup waterer to allow ad libitum consumption of feed and water.

Experimental diets were fed for 17 d in Exp. 1 and 21 d in Exp. 2, and were fed in meal form in both experiments (Table 1). Diets were formulated to meet or exceed the nutrient requirements of pigs for all nutrients except lysine and threonine. Ingredient nutrient compositions and TID coefficients provided by the NRC were used in diet formulation. Pigs were weighed and feed disappearance was measured on d 7, 14, and 17 (Exp. 1), and every 7 d (Exp. 2) to determine ADG, ADFI, and F/G.

**Experiment 1.** Three hundred and sixty barrows and gilts ( $16 \pm 2$  d of age at weaning) were blocked by weight at 18 d postweaning, and were allotted randomly to one of nine dietary treatments. Each treatment had eight rep-

lications (pens) per treatment, with five pigs per pen.

For the first 14 d postweaning, pigs were fed a diet containing 20% spray-dried whey and 1.51% total lysine; a diet containing 1.4% lysine was fed from d 14 to 18. Pigs were fed experimental diets from d 18 to 35 postweaning (23.6 to 45.2 lb). The first part of the trial consisted of five treatments formulated to contain 0.9, 1.0, 1.1, 1.2, or 1.3% TID lysine, with TID threonine at 66% of lysine. The second part consisted of five treatments formulated to 1.3% TID lysine, with increasing TID threonine (0.60, 0.66, 0.73, 0.79, or 0.85%). Cornstarch replaced L-lysine or L-threonine in the control diets to form the dietary treatments. The diets containing 0.9% TID lysine with 0.60% TID threonine, 1.3% TID lysine with 0.60% TID threonine, and 1.3% TID lysine with 0.85% TID threonine were blended to form all other diets.

Blood samples were obtained by venipuncture on d 12 from two randomly selected pigs in each pen after a 3-h period of feed deprivation. All samples were centrifuged ( $13,800 \times g$ ) before further preparation. Plasma urea N (PUN) determination was performed on each sample by using an autoanalyzer. Plasma from pigs in the same pen was pooled and prepared by using an EZ:faast™ Amino Acid Analysis Kit (Phenomenex®, Torrance, CA) for gas chromatographic analysis.

**Experiment 2.** One thousand, four-hundred and forty barrows and gilts ( $18 \pm 2$  d of age at weaning) were blocked by gender at 21 d postweaning, and were allotted randomly by pen to one of five dietary treatments. Two pens shared the same feeder, with feeder as the experimental unit. Thus, there were 48 pigs per experimental unit and six replications per treatment. Pigs were fed experimental diets from d 21 to 42 postweaning (24 to 44.5 lb).

Immediately postweaning, all pigs were fed 1.1 lb of a complex diet containing spray-dried animal plasma, fishmeal, spray-dried blood cells and spray-dried whey with 1.69% total lysine, followed by 4 lb of a less complex diet containing 1.55% lysine. The diet containing 1.55% lysine was then fed until initiation of the experiment. All diets were formulated to contain 1.1% TID lysine, which is less than the requirement of 1.4% TID lysine previously established for pigs in this facility. L-threonine was added to provide 0.55, 0.60, 0.66, 0.72 or 0.77% TID threonine. The negative- and positive-control diets containing 0.55 and 0.77% TID threonine, respectively, were blended to form the diets of 0.60, 0.66, and 0.72% TID threonine. Subsamples of each dietary treatment were analyzed for amino acid content, and were within expected analytic variation of expected values.

**Statistical Analysis.** In Exp. 1, data was analyzed as a randomized complete-block design with pen as the experimental unit. Pigs were blocked on the basis of weight at d 18 postweaning, and analysis of variance was performed by using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Linear and quadratic polynomial contrasts were performed to determine the effects of increasing dietary lysine and threonine.

In Exp. 2, data were analyzed as a randomized complete-block design by using the PROC MIXED procedure of SAS, with feeder used as the experimental unit. Linear and quadratic polynomial contrasts were performed to determine the effects of increasing dietary threonine. A feeder from each of the 0.55, 0.60, and 0.72% treatments was dropped from the analysis due to an unrelated health event that greatly reduced the growth rate of pigs in these pens. Weight of pigs at the start of the experiment on d 21 post-weaning was used as a covariate in the analysis.

## Results

### *Experiment 1*

**Lysine Trial.** Overall, there was an increase in ADG (quadratic  $P<0.02$ ) as dietary lysine increased from 0.9 to 1.3% TID lysine, with the greatest ADG observed at 1.2% TID lysine (Table 2). Feed efficiency improved (quadratic,  $P<0.01$ ) as TID lysine increased to 1.2%, and then F/G plateaued. Plasma urea N, measured on d 12, decreased (linear,  $P<0.0001$ ) as TID lysine increased to 1.2% (Table 3). Plasma lysine concentrations increased (linear,  $P<0.01$ ) with increasing TID lysine. Plasma histidine, isoleucine, phenylalanine, and valine concentrations decreased (linear,  $P<0.05$ ) as TID lysine increased, but plasma methionine increased (linear,  $P<0.05$ ) as TID lysine increased. No changes in other amino acid concentrations were observed as TID lysine increased.

**Threonine Trial.** Increasing TID threonine increased (linear,  $P<0.03$ ) ADG (Table 2). Feed efficiency also improved (quadratic,  $P<0.04$ ) as TID threonine increased from 0.60% to 0.79%, and F/G did not improve thereafter. Plasma urea N concentration decreased (linear,  $P<0.04$ ) as TID threonine increased (Table 3). Plasma threonine concentration increased (quadratic,  $P<0.01$ ) with increasing TID threonine, but plasma lysine concentrations were unchanged. Plasma isoleucine (linear,  $P<0.02$ ), valine (linear,  $P<0.05$ ) and tyrosine (quadratic,  $P<0.05$ ) concentrations increased as TID threonine increased to 0.79%, then decreased at 0.85% TID threonine.

Average daily gain and F/G improved with increasing TID lysine up to 1.2%, and up to 0.79% TID threonine. Regression analysis revealed that 95% or more of the maximum response was obtained at a TID threonine-to-lysine ratio of approximately 64% for ADG and 66% for F/G. The results suggest that the TID threonine-to-lysine ratio for 24- to 44-lb pigs is approximately 64% to 66%.

### **Experiment 2**

Overall, there was an increase in ADG (quadratic,  $P < 0.07$ ) as TID threonine increased from 0.55 to 0.72% (Table 4). Feed efficiency also improved (quadratic,  $P < 0.01$ ) with increasing TID threonine. With a lysine concentration of 1.1% TID lysine, and a TID threonine requirement of 0.72%, a TID threonine-to-lysine ratio of 65% was determined.

### **Discussion**

The NRC currently suggests a TID threonine-to-lysine ratio of 62% for a 24- to 44-lb pig. This estimate is derived from many trials that investigated the optimal threonine-to-lysine ratio by titrating different threonine concentrations in diets containing a predetermined lysine concentration; this approach is similar to the approach used in our second experiment. Other trials have examined lysine and threonine requirements separately. There are potential problems with this approach to determine a ratio, because a certain lysine concentration is chosen without knowledge of the actual lysine requirement for the specific group of pigs used in the various studies. Therefore, extrapolating a ratio for an amino acid relative to lysine can be biased by the lysine concentration assumed to be the requirement. Experiment 1 was run as a combination of two separate trials in which the TID lysine and TID threonine requirements were examined simultaneously to determine the threonine-to-lysine ratio. This approach to determining an optimal ratio offers greater accuracy because lysine and threonine requirements for a group of pigs are determined simultaneously.

Experiment 1 suggests a TID lysine requirement of 1.2% for a 24- to 44-lb pig. This requirement is greater than the TID lysine requirement of 1.01% suggested by the NRC, but less than the requirement found in recent experiments at the University of Missouri and Kansas State University. Genotype and envi-

ronment potentially alter the maintenance requirement and change the absolute amount of lysine needed for maximum growth. The pigs used in the University of Missouri and Kansas State University trials were PIC, whereas our study was conducted with Genetiporc pigs. This may explain the difference in the TID lysine requirement suggested by the previous studies (PIC) compared with that in our experiment (Genetiporc). In Exp. 2, the lysine concentration was set at 1.1% TID lysine, and was less than the actual requirement (1.4%) that had been previously determined for pigs in these facilities.

The arrangement of Exp. 1 as a combination trial that determined both the lysine and threonine requirements simultaneously allowed the use of a regression approach in the establishment of a TID threonine-to-lysine ratio. In this approach, ADG and F/G values are plotted as the dependent variables on the x axis with the TID lysine and threonine concentrations on the Y-axis (Figures 1 to 4). A trend line is fit through the data to develop a regression equation to predict the TID lysine and threonine requirement; the trend line can be used to estimate the TID threonine-to-lysine ratio. The values for ADG and F/G from the individual lysine and threonine trials must overlap to allow this approach to work. In our trial, ADG is shown to be maximized at a threonine-to-lysine ratio approaching 70% (Table 5). But almost 97% of the maximum response can be achieved using a TID threonine-to-lysine ratio of approximately 64%. Feed efficiency was optimized at a TID threonine-to-lysine ratio of 66% (Table 5). This analysis further verifies a TID threonine-to-lysine ratio of approximately 66%, as suggested by our performance and blood data in Exp. 1 for the 24- to 44-lb pig.

The PUN and individual amino acid concentrations in plasma are consistent with the typical responses expected when titrating limiting amino acids. When the concentration of the test amino acid is less than the require-

ment, PUN values are high as a result of oxidation of plasma amino acids because protein synthesis is limited by the test amino acid. As the concentration of the test amino acid approaches and equals the requirement, PUN values decrease as other amino acids are incorporated into protein synthesis, and are no longer oxidized. But the concentration of the test amino acid in plasma increases as it is no longer limiting and begins to exceed the requirement for protein synthesis.

From the lysine and threonine estimates suggested by the NRC, a TID threonine-to-lysine ratio of 62% (total threonine-to-lysine ratio of 64%) is implicated. When compared on a ratio basis, the 64 to 66% TID threonine-to-lysine ratio suggested by our trials is slightly greater than the 62% suggested by the NRC.

**Table 1. Composition of Diets (As-fed Basis)**

Ingredient, %	Experiment 1 <sup>a</sup>	Experiment 2 <sup>b</sup>
Corn	65.96	71.13
Soybean meal, 46.5% CP	27.65	23.60
Soy oil	1.50	---
Choice white grease	---	1.00
Monocalcium phosphate, 21% P	1.55	---
Dicalcium phosphate, 18.5% P	---	1.40
Limestone	0.95	0.75
Salt	0.35	0.35
Vitamin/trace mineral premix	0.40	0.30
Medication	0.50	0.70
L-lysine HCl	0.53	0.40
L-threonine	0.25	0.23
DL-methionine	0.23	0.14
L-valine	0.08	---
Tryptophan	0.03	---
L-isoleucine	0.02	---
Total	100.0	100.0
True-ileal-digestible lysine, %	1.30	1.10
True-ileal-digestible threonine, %	0.85	0.77
Isoleucine:lysine ratio, %	55%	58%
Leucine:lysine ratio, %	116%	129%
Methionine:lysine ratio, %	38%	34%
Met & Cys:lysine ratio, %	60%	60%
Threonine:lysine ratio, %	66%	71%
Tryptophan:lysine ratio, %	17%	16%
Valine:lysine ratio, %	65%	67%
ME, kcal/kg	3,355	3,344
Crude protein, %	18.5	17.0
Ca, %	0.76	0.68
P, %	0.70	0.62
Available P, %	0.40	0.32
TID lysine:calorie ratio, g/mcal	3.87	3.29

<sup>a</sup>In Exp. 1, cornstarch replaced L-lysine or L-threonine in the control diet to provide 0.9, 1.0, 1.1, 1.2, or 1.3% TID lysine and 0.60, 0.66, 0.73, 0.79, or 0.85% TID threonine for the dietary treatments. Analyzed values for total lysine were 1.06, 1.16, 1.25, 1.32, and 1.39 %, respectively, in the first part of the trial, and analyzed total threonine values were 0.78, 0.80, 0.88, 0.91, and 0.96%, respectively for the second part of the trial. The diets containing 0.9% TID lysine with 0.60% TID threonine, 1.3% TID lysine with 0.60% TID threonine, and 1.3% TID lysine with 0.85% TID threonine were blended to form all other diets.

<sup>b</sup>In Exp. 2, analyzed total threonine values were 0.68, 0.70, 0.66, 0.77, and 0.83%. The negative- and positive-control diets containing 0.55 and 0.77% TID threonine, respectively, were blended to form the diets with 0.60, 0.66, and 0.72% TID threonine.

**Table 2. Effects of Increasing True-ileal-digestible (TID) Lysine and Threonine on Growth Performance of 24- to 44-lb Nursery Pigs (Exp. 1)<sup>a</sup>**

Item	TID Lysine, %					TID Threonine, %					SED	Probability (P<)			
	0.9	1.0	1.1	1.2	1.3 <sup>b</sup>	0.60	0.66	0.73	0.79	0.85 <sup>b</sup>		Lysine		Threonine	
												Linear	Quadratic	Linear	Quadratic
ADG, lb	1.17	1.19	1.29	1.32	1.28	1.24	1.26	1.27	1.33	1.28	0.04	0.01	0.02	0.03	0.26
ADFI, lb	2.02	1.92	2.02	2.03	1.98	2.04	1.98	1.98	2.03	1.98	0.08	0.81	1.00	0.45	0.64
F/G	1.72	1.61	1.56	1.54	1.54	1.64	1.57	1.56	1.53	1.54	0.02	0.01	0.01	0.01	0.04

<sup>a</sup> A total of 360 pigs (average wt of 23.6 lb), with five pigs per pen and eight pens per treatment, with experimental diets fed for 17 d.

<sup>b</sup> The diets containing 1.3% TID lysine and 0.85% TID threonine were combined as one treatment, giving a total of eight replications.

**Table 3. Effects of Increasing True-ileal-digestible (TID) Lysine and Threonine on Plasma Amino Acid Profile and Plasma Urea Nitrogen of the 22- to 44-lb Nursery Pig (Exp. 1)<sup>a</sup>**

Amino acid, µm/L	TID Lysine, %					TID Threonine, %					SED	Probability (P<)			
	0.9	1.0	1.1	1.2	1.3	0.60	0.66	0.73	0.79	0.85		Lysine		Threonine	
												Linear	Quadratic	Linear	Quadratic
Lysine	58	85	90	143	154	212	170	179	209	154	15.78	0.01	0.78	0.38	0.64
Threonine	230	223	175	223	206	62	70	131	243	206	16.85	0.38	0.23	0.01	0.01
Histidine	65	48	42	35	34	40	31	34	42	34	6.96	0.01	0.22	0.40	0.77
Isoleucine	103	103	92	94	88	86	86	85	97	88	3.41	0.01	0.93	0.02	0.16
Leucine	178	201	175	176	169	176	171	176	192	169	8.30	0.11	0.30	0.26	0.18
Methionine	27	30	35	33	49	37	27	34	44	49	7.41	0.05	0.49	0.08	0.81
Phenylalanine	88	83	75	71	71	75	61	71	77	71	3.85	0.01	0.40	0.10	0.35
Tryptophan	47	47	42	46	43	43	41	42	48	43	2.85	0.27	0.80	0.12	0.33
Tyrosine	97	105	91	100	87	94	91	101	108	87	6.63	0.26	0.45	0.63	0.05
Valine	208	195	170	154	166	179	154	164	190	166	10.21	0.01	0.14	0.05	0.44
PUN, mg/dL	21.9	15.51	15.46	8.74	13.92	15.52	8.32	9.87	12.63	13.92	1.75	0.01	0.01	0.04	0.19

<sup>a</sup> Values represent the mean of eight replications (pens) of individual samples from two pigs per pen for plasma urea nitrogen concentration and pooled samples from two pigs per pen for plasma amino acid concentrations. Blood samples were collected on d 12, after 3 h feed withdrawal.

**Table 4. Effect of Increasing True-ileal-digestible (TID) Threonine on Growth Performance of 24- to 45-lb Nursery Pigs (Exp. 2)<sup>ab</sup>**

Item	TID Threonine, %					SED	Probability (P<)	
	0.55	0.60	0.66	0.72	0.77		Linear	Quadratic
ADG, lb	0.93	0.99	0.99	1.01	1.00	0.021	0.001	0.06
ADFI, lb	1.71	1.76	1.69	1.72	1.71	0.038	0.53	0.82
F/G	1.84	1.79	1.72	1.70	1.71	0.021	0.001	0.01
Avg weight, lb d 42	43.7	45.0	44.9	45.5	45.2	0.46	0.002	0.06

<sup>a</sup>A total of 1,440 pigs (average wt of 24.0 lb), with 48 pigs per experimental unit and six replications per treatment, with experimental diets fed for 21 d.

<sup>b</sup>Diets contained 1.1% TID lysine to give TID threonine-to-lysine ratios of 50, 55, 60, 65, and 70%.

**Table 5. Estimation of True-ileal-digestible (TID) Lysine and Threonine Requirements, and Threonine-to-lysine Ratio, Based on Regression Analysis for Different Levels of Pig Performance (Exp. 1)<sup>a</sup>**

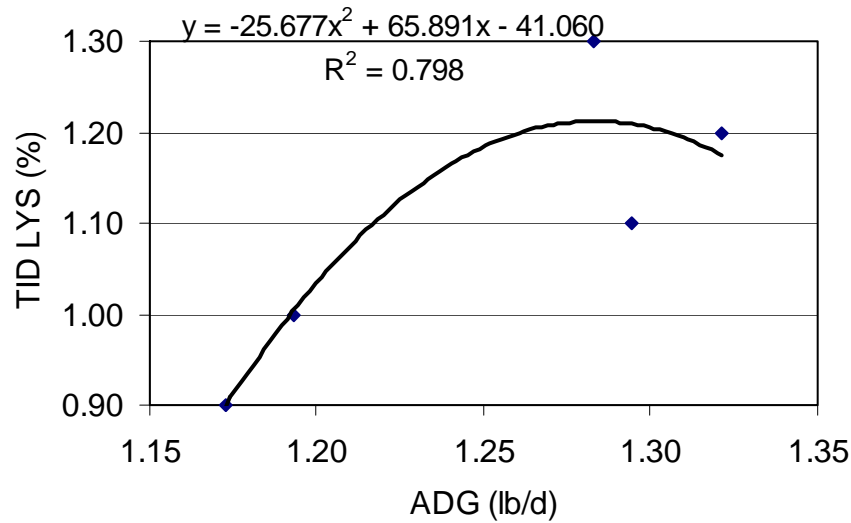
Item	Lysine, %	Threonine, %	Threonine:Lysine	% of Maximum
ADG, lb <sup>b</sup>				
1.24	1.164	0.571	49.0	93.9
1.27	1.207	0.748	61.9	96.2
1.30	1.204	0.824	68.4	98.5
1.32	1.177	0.818	69.5	100.0
F/G <sup>c</sup>				
1.64	0.915	0.599	65.5	93.7
1.60	1.001	0.610	61.0	96.0
1.56	1.140	0.707	62.1	98.5
1.54	1.248	0.807	64.7	100.0

<sup>a</sup>The range of ADG and F/G values as observed in Exp. 1 were plotted against TID lysine and threonine concentrations used in the experiment to determine TID lysine and threonine concentrations necessary to achieve a given ADG or F/G, and hence, a TID threonine-to-lysine ratio.

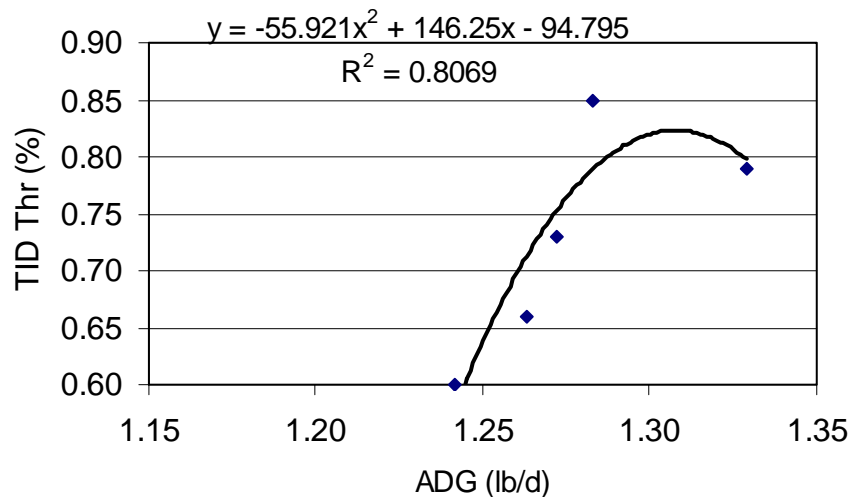
<sup>b</sup>Regression equations of  $y = -25.677x^2 + 65.891x - 41.06$  and  $y = -55.921x^2 + 146.25x - 94.795$  were used to determine lysine and threonine requirements, respectively, for the range of ADG values (Figures 1 and 2).

<sup>c</sup>Regression equations of  $y = 16.396x^2 - 55.278x + 47.472$  and  $y = 26.919x^2 - 87.494x + 71.688$  were used to determine lysine and threonine requirements, respectively, for the range of F/G values (Figures 3 and 4).

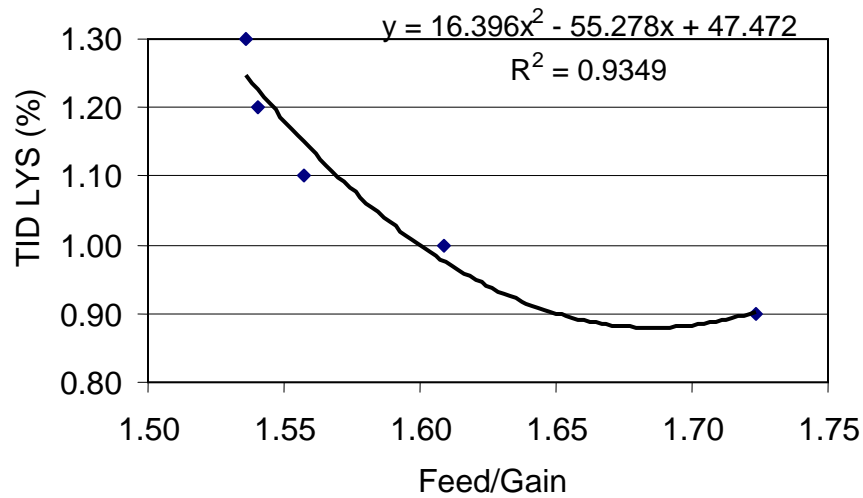




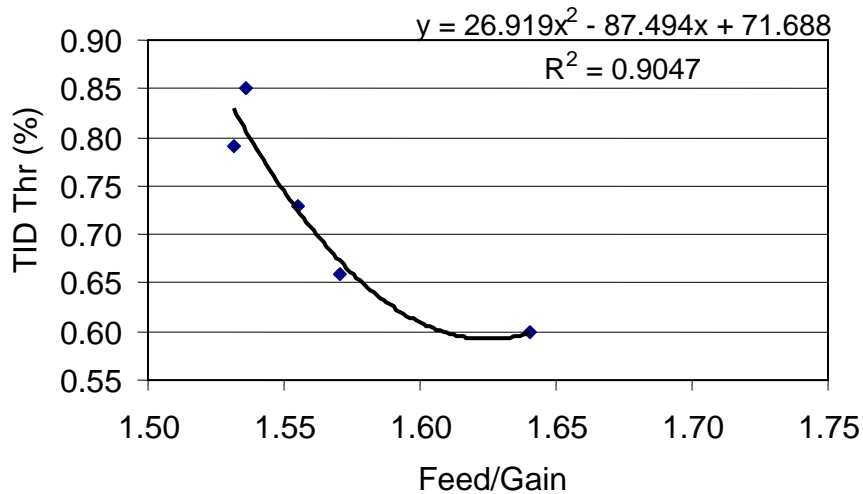
**Figure 1. Effect of Increasing True-ileal-digestible Lysine on Average Daily Gain of Nursery Pigs (Exp. 1).** A total of 360 pigs (average wt of 23.8 lb), with five pigs per pen and eight pens per treatment, with experimental diets fed for 17 d. True-ileal-digestible lysine concentrations were 0.9, 1.0, 1.1, 1.2, and 1.3%. The ADG values were plotted against TID lysine concentrations used in the experiment to determine the lysine concentration necessary to achieve a certain average daily gain.



**Figure 2. Effect of Increasing True-ileal-digestible Threonine on Average Daily Gain of Nursery Pigs (Exp. 1).** A total of 360 pigs (average wt of 23.8 lb), with five pigs per pen and eight pens per treatment, with experimental diets fed for 17 d. True-ileal-digestible threonine concentrations were 0.60, 0.66, 0.73, 0.79, and 0.85%. The ADG values were plotted against TID threonine concentrations used in the experiment to determine the threonine concentration necessary to achieve a certain average daily gain.



**Figure 3. Effect of Increasing True-ileal-digestible Lysine on Feed Efficiency of Nursery Pigs (Exp. 1).** A total of 360 pigs (average wt of 23.8 lb), with five pigs per pen and eight pens per treatment, with experimental diets fed for 17 d. True-ileal-digestible lysine concentrations were 0.9, 1.0, 1.1, 1.2, and 1.3%. The F/G values were plotted against TID lysine concentrations used in the experiment to determine the lysine concentration necessary to achieve a certain F/G ratio.



**Figure 4. Effect of Increasing True ileal digestible Threonine on Feed Efficiency of Nursery Pigs (Exp. 1).** A total of 360 pigs (average wt of 23.8 lb), with five pigs per pen and eight pens per treatment, with experimental diets fed for 17 d. True-ileal-digestible threonine concentrations were 0.60, 0.66, 0.73, 0.79, and 0.85%. The F/G values were plotted against TID concentrations used in the experiment to determine the threonine concentration necessary to achieve a certain F/G ratio.